

Date: 11/2/13 Making Competent Cells

Persons participating: Levi Palmer, Emily Puleo, Kelsey Crossen

- 9am - Will make chemically competent DH5a cells.

Date: 11/2/13 People in lab: Levi Palmer

Title: Test 11/2 competent cells for competence

Start Time: 5:48 PM

Purpose: To test for competence of cells by transformation

Protocol: LTM ed. 2

Products:

Sample Label	Description	Source Label	Quantity
11/2/13 G1 control CT	11/2 competent cells with pUC19, not grown on antibiotic, positive control	11/2 G1 pUC19 CT	4
11/2/13 G1 pUC19 CT	11/2 competent cells with pUC19, grown on Ampicillin	11/2 G5 pUC19 CT	4

Results: Unsure results, lawn growth, retest with negative control

Stop Time: 9:00 PM

Date: 11/08/13 **People in lab:** Levi Palmer

Title: Chemical Transformation test for competency

Start Time: 6:00 PM

Purpose: To test 11/1/13 Chemically Competent cells for competency.

Protocol: iGEM LTM ed. 2 Pages: 41-43 Exceptions: 1. Only used 1 μ L of pUC19 plasmid. 2. Negative and positive controls were made.

Products:

Sample Label: CT pUC19 11/8/13 1 Description: Cells transformed with pUC19 Source Label: pUC19, Chem. Comp. Cells 11/1/13

Sample Label: CT pUC19 11/8/13 2 Description: Cells transformed with pUC19 Source Label: pUC19, Chem. Comp. Cells 11/1/13

Sample Label: Negative control Description: Untransformed cells Source Label: Chem. Comp. cells 11/1/13

Sample Label: Positive control Description: Cells transformed with pUC19, no Amp. Source Label: pUC19, Chem. Comp. Cells 11/1/13

Results: Plates did not have enough ampicillin, negative control grew.

Notes:

Stop Time: 9:00 PM

Next: Make more amp plates.

Date: 11/18/13 People in lab: Blythe Ferriere

Title: PCR norV for PstI digestion

Start Time: 9:00 AM

Purpose: To amplify the norV gene before PCR mutagenesis to remove the PstI site

Protocol: LTM ed. 2

Products:

Sample Label	Description	Quantity
11/18/13 norV A	norV with iGEM prefix/suffix	2

Notes: Used norCB program on PCR machine

Stop Time: 3:00 PM

Next: Digest with PstI

Date: 11/19/13 People in lab: Blythe Ferriere

Title: Digest 11/18 norV with PstI

Start Time: 9:09 AM

Purpose: To digest the PCR product from 11/18 to select against the fragments with the PstI site

Protocol: LTM ed. 2

Products:

Sample Label	Description	Source Label	Quantity
11/20/13 D1 norV	norV PCR product digested with PstI	11/18 norV A	2

Stop Time:

Date: 11/24/13 People in lab: Blythe Ferriere

Title: PCR of PstI digested norV, and then gele electrophoresis of PCR

Start Time: 12:40 PM

Purpose: To use the digested PCR product as template for new PCR, fragments of correct size on gel won't have PstI cut site

Protocol: LTM ed. 2 Exceptions: Template DNA used was 11/20 D

Products:

Sample Label	Description	Source Label	Quantity
11/24/13 norV A1	norV gene and fragments, amplified	11/20 D1	2

Well	1	2	3	4	5	6	7	8
Sample	Ladder	A1	Ladder	A2				

Results:

Stop Time:

Date: 11/25/13 People in lab: Blythe Ferriere

Title: PCR norV for PstI digest and gel electrophoresis/extraction, then digest with PstI

Start Time: 12:15 PM

Purpose: To perform the steps necessary for site-directed PCR mutagenesis

Protocol: LTM ed. 2

Products:

Sample Label	Description	Source Label	Quantity
11/25/13 norV A1	norV gene with prefix and suffix	E. coli 7/31	2
11/25/13 norV A3	Control to make sure PCR from a PCR works, norV from norV	11/25/13 norV A1	2
11/25/13 norV A5	PCR of norV with shorter annealing time	E. coli 7/31	2
11/25/13 GE 1	Extraction of A5	11/25/13 norV A5	2
11/25/13 norV A7	PCR of extracted PCR products	11/25/13 GE1	2
11/25/13 D1	Digestion of A7 with PstI	11/25/13 norV A7	2

Well	1	2	3	4	5	6	7	8
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Sample	Ladder	A1		Ladder	A2			
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Well	1	2	3	4	5	6	7	8
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Sample	Ladder	A5		Ladder	A6			
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Well	1	2	3	4	5	6	7	8
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Sample	Ladder	A7		Ladder	A8			
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Results:

Notes: Used norCB program, used water to elute DNA from gel purification

Stop Time:

Next: